Denaturation of Cottage Cheese Whey Proteins by Heat

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Abstract

The proteins in Cottage cheese whey were comparatively stable to heat; thirty minutes at 91 C were required to denature 80% of the proteins, whereas 81 C for 30 min denatured the same amount of protein in skimmilk.

The Harland-Ashworth test provided a reasonably accurate index to the extent of heat denaturation, if the pH of the whey was adjusted to 5.4-6.5 immediately prior to analysis. The observed whey protein stability did not result from the presence of lactate ion, but resulted from the low pH of the whey.

No significant increase in heat denaturation rate was obtained by concentrating the whey prior to heating. Maximum heat stability was usually observed in whey concentrates containing 20% total solids. During heating, the buffering capacity of the Cottage cheese whey was reduced.

During a study of possible uses for a recently developed Cottage cheese whey powder (2), a question arose as to the heat stability of the proteins in acidic Cottage cheese whey.

Although published data relating the pH of solutions of β-lactoglobulin to their heat stability (4) were indicative, no published work describing denaturing by heat of proteins in Cottage cheese whey could be found. Results obtained in our investigation are presented here.

Our paper contains descriptions of simple methods for the determination of the extent of heat denaturation of proteins in Cottage cheese whey and data pertaining to factors influencing this phenomenon.

Materials and Methods[1]

Whey preparation. Cottage cheese whey was made from skimmilk pasteurized by holding at 63 C for 30 min. On cooling to 32 C, lactic starter was added. After 1 hr of incubation 8 ml of a 1%-rennet solution per 3.78 liters of milk were added and incubation continued for an additional 3 hr. The mixture was then cooked for 1 hr at 49-50 C and the whey then drained off for study.

All milks used for comparative purposes were pasteurized under the same conditions and represent a source common to the whey.

Wheys were also obtained, in small amounts, by centrifuging milk at 105,000 *g* (avg) for 90 min or by precipitating casein with acids added to pH 4.6.

Cottage cheese whey concentrates were prepared by evaporation under reduced pressure in an all-glass rotating evaporator of custom design. Concentrates having total solids (TS) content of approximately 50% were diluted with distilled water to provide samples having a variety of TS content.

Denaturation procedures. All heat denaturation of proteins in single-strength wheys and milks was done by heating 75-ml aliquots for 30 min in 125-ml Erlenmeyer flasks suspended in a Magna Whirl water bath set at the desired temperature. At the end of the heating period, the flask and contents were cooled to 21 C in an ice-water bath. Proteins in concentrated wheys were denatured by placing 25-ml aliquots in 190- by 30-mm test tubes and handling them as described. After heating, concentrated wheys were diluted to a 6.7% TS level for analysis.

Determination of extent of whey protein denaturation. The extent of whey protein denaturation effected by the various heat treatments used in this study was measured by:

a) Leighton's (3) modification of the Harland-Ashworth procedure as described for nonacid wheys. When applied to Cottage cheese whey or other acid wheys, the pH was adjusted to 6.0-6.5 by addition at most of 0.4% volume of 40% NaOH prior to running the test.

b) Kjeldahl determination of residual nitrogen in the supernatants of wheys adjusted to pH 4.6 and centrifuged at 78,000 *g* (avg) for 30 min. Cottage cheese whey did not require acidification before centrifugation. A comparison of these values with those obtained in similar fashion, using an unheated control, allowed calculation of the per cent whey protein denaturation. Corrections for nonprotein in the whey were made in all instances.

c) Fluorimetric determination of residual proteins obtained as described above. Aliquots

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[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

of the supernatants were diluted 1:100 with distilled water and exposed to 280 mμ radiation. The fluorescence at 340 mμ was measured and converted to protein concentration as described by Fox et al. (1).

Corrections for nonprotein nitrogen fluorescence were made by observing the residual fluorescence in samples of the supernatants from which the proteins had been removed by trichloroacetic acid precipitation. The trichloroacetic acid was removed by ether extraction before fluorimetric analysis.

Titration of Cottage cheese whey. Changes in the titration curve of Cottage cheese whey resulting from heat treatment of the product were studied, using a Radiometer Titrator and Titrograph-equipped microburette. The samples were cleaned of suspended material by centrifugation. A 0.3-ml aliquot of the supernatant was placed in the titration cell, 10 ml of CO_2-free distilled water added, and the titration carried out using 0.0256 N NaOH. The change in pH by adding base was recorded automatically.

Results

The effect of shifting the pH of Cottage cheese whey before analysis for undenatured whey proteins, by the modified Harland-Ashworthprocedure (3), is shown in Figure 1. From this graph it can be seen that the pH of Cottage cheese whey should be adjusted to approximately 6.0, if accurate determinations are to be achieved.

When the pH of the Cottage cheese whey is adjusted to approximately 6.0 before analysis by the modified Harland-Ashworth method results obtained in heat denaturation studies compare favorably with those obtained by more direct methods, especially by heating above 85 C for 30 min (Figure 2). Here the per cent whey proteins in Cottage cheese whey denatured by heating 30 min from 72 to 94 C are shown as determined by three different methods.

The proteins in Cottage cheese whey are relatively resistant to heat denaturation (Figure 3). Here the whey protein denatured by heating skimmilk, whey produced by ultra-centrifugation, and Cottage cheese whey are compared.

On adjusting the pH of the Cottage cheese whey upward, prior to heating, the observed heat stability of the whey proteins was lost (Figure 4).

The fact that the observed stability of the Cottage cheese whey proteins resulted from the low pH of the product and was not brought

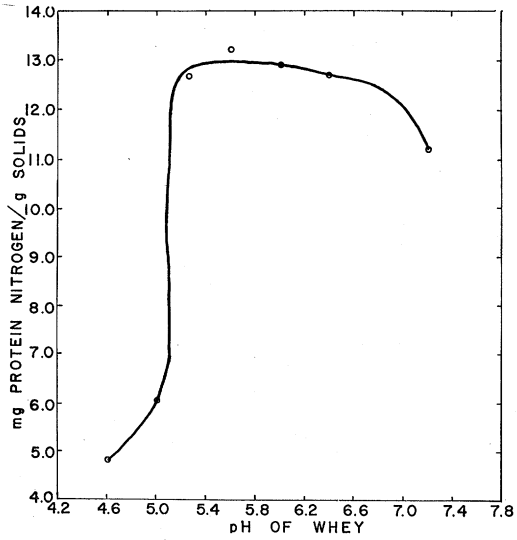

FIG. 1. Effect of adjusted pH of Cottage cheese whey, prior to analysis, on results obtained by the modified Harland-Ashworth test.

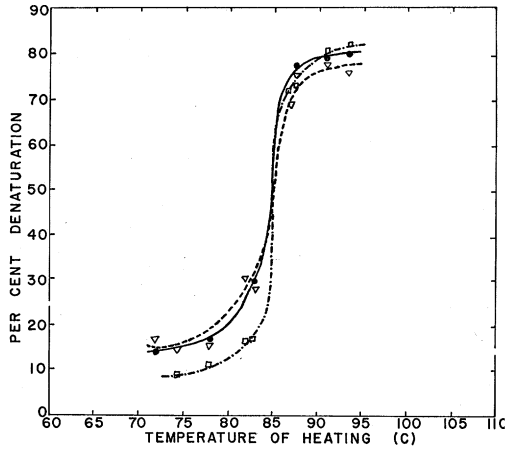

FIG. 2. Rate of protein denaturation in Cottage cheese whey as measured by three different methods: Kjeldahl, triangles; fluorimetric, circles; modified Harland-Ashworth with pH adjusted to 6.0-6.5 prior to analysis, squares.

TABLE 1

Effect of heat on denaturation of acid wheys prepared with different acidulants

Temp (C)	% Denaturation by the Kjeldahl procedure		
Heating 30 min	HCl	Lactic acid whey	Cottage cheese whey
72	15.1	18.9	16.8
78	17.1	19.2	15.2
83	27.0	29.0	27.7
85	52.8	56.5	54.5
88.5	72.7	73.4	74.8

bout, at least in part, by fermentation products in the whey, is established by the data in Table 1. Here, no significant differences are noted between the heat stability of the proteins found in wheys produced by direct acidification with HCl or lactic acid and indirect acidification by fermentation—the final pH of all systems being 4.6.

In view of certain commercial advantages that could accrue from carrying out heat treatment on condensed Cottage cheese whey, the effect of the total solids content of the whey on its protein stability to heat denaturation was studied. Results are summarized in Figure 5. Here, rather unusual behavior of the whey proteins is noted. At 87 C the amount of whey protein denatured in 30 min of holding reaches a minimum value at concentrations of 20% TS. At 87 C, the same amount of heat denaturation occurs in the 40% TS concentrate and the single-strength whey. On heating at 84.5 C for 30 min, a minimum in denaturation values is observed at 20% total solids in the wheys. However, more denaturation is observed in the 40% TS concentrate than in the single-strength whey. These results do not stem from a pH shift during concentration, since a sequential rise of only .18 pH unit was noted as the TS of the whey rose to 40%.

In general, heat treatment caused slight upward shifts in the pH of the wheys. This is shown in Figure 6, where the effect of added alkali on the pH of wheys subjected to various heat treatments is shown. These titration curves demonstrate that the buffering capacity of Cottage cheese whey decreases with increased severity of heat treatment.

Discussion

The observed fact that the proteins in Cottage cheese whey are more resistant to heat denaturation than similar proteins in milk is in agreement with results obtained by Mularz and

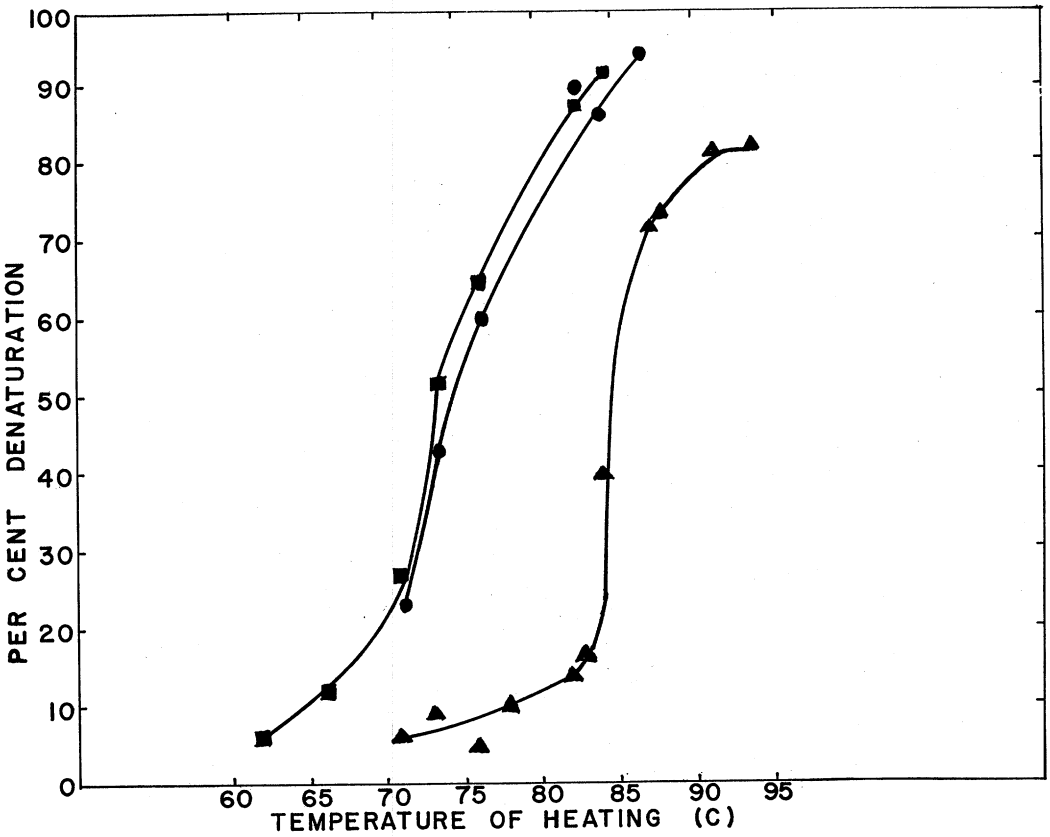

FIG. 3. The comparative rates of protein denaturation in skimmilk, Cottage cheese whey, and whey prepared by removing casein from skimmilk by centrifuging in a Spinco preparative ultracentrifuge at 105,000 *g* (avg) for 90 min. Measurements made using the modified Harland-Ashworth procedure: skimmilk, squares; ultracentrifuged whey, circles; Cottage cheese whey, triangles. All samples heated for 30 min at indicated temperatures.

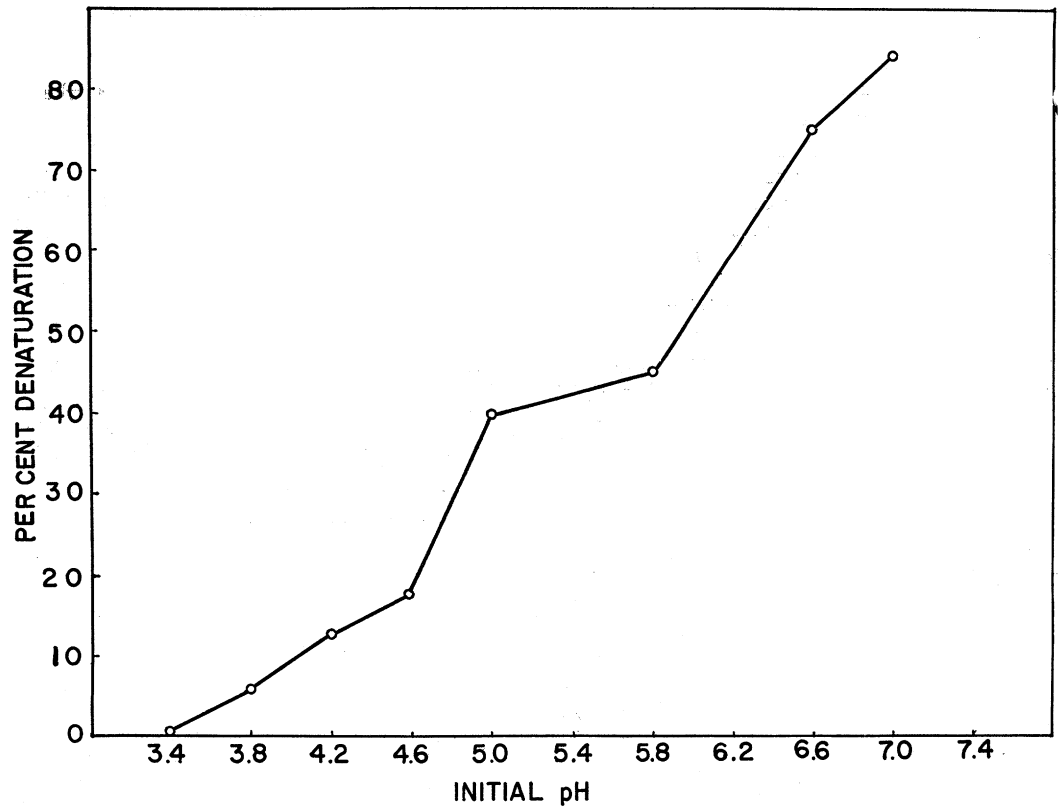

FIG. 4. The effect of pH adjustment prior to heating at 82 C for 30 min on the denaturation of Cottage cheese whey proteins as measured by the modified Harland-Ashworth procedure.

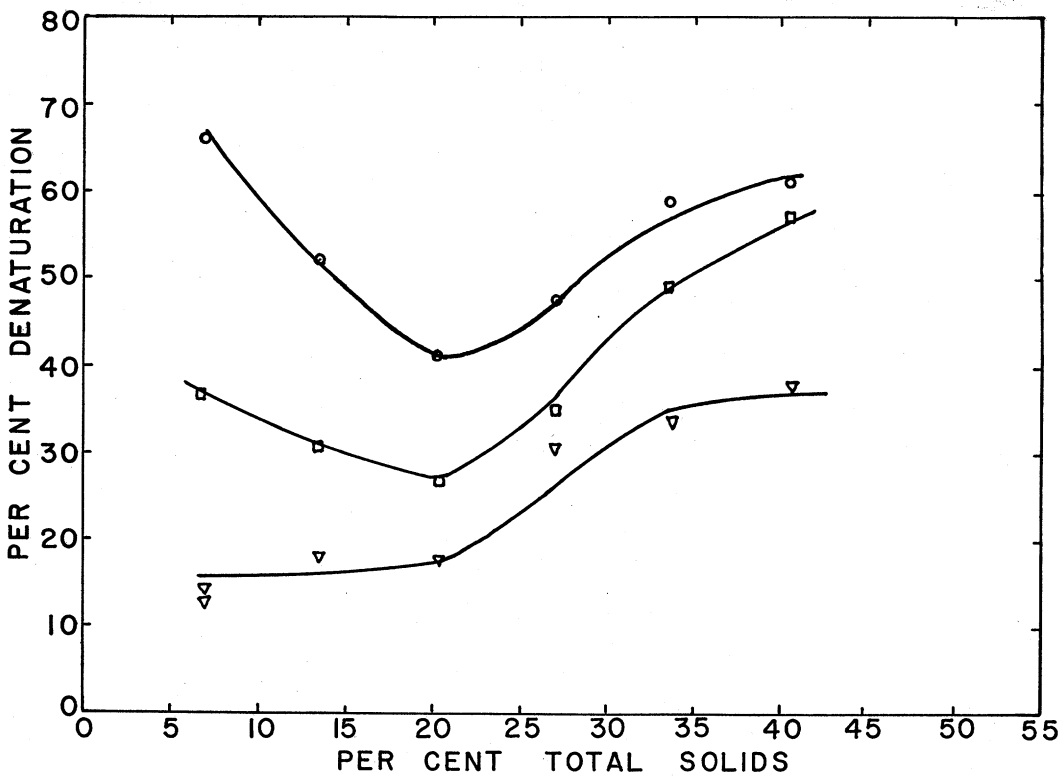

FIG. 5. The effect of total solids content of Cottage cheese whey concentrates on the amount of protein denatured by heating at 87 C for 30 min, circles; 84.5 C for 30 min, squares; 74 C for 30 min, triangles. Kjeldahl procedure used.

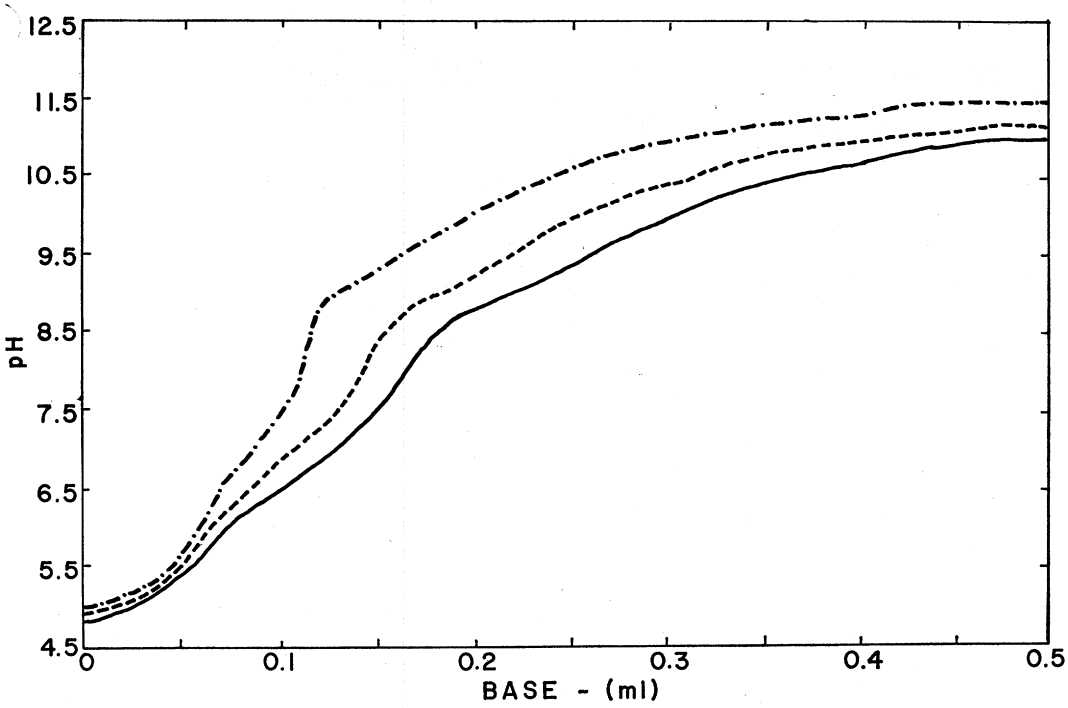

FIG. 6. The titration curves of Cottage cheese whey subjected to various heat treatments; unheated, solid line; 74 C for 30 min, broken line; 85 C for 30 min, dot-dash.

Swanson (4) in their studies of the heat stability of β-lactoglobulin.

Although shifting the pH of Cottage cheese whey upward prior to heating could be used to increase the rate of protein denaturation, no similar simple effect could be observed on increasing the total solids in the whey prior to heating. The reasons for the occurrence of maximum protein stability at 20% TS concentration are undergoing investigation.

Since the buffering capacity of the Cottage cheese whey is not high, the further loss of capacity on heating may furnish the basis for a quick test for the extent of heat-induced change in the whey system. The addition of an appropriate amount of base to the whey, followed by pH measurement, should result in higher readings for heated whey than those observed using unheated whey.

Even though the protein fraction in Cottage cheese whey is heterogeneous, the shape of the curve describing the relationship between pH and amount of whey protein denaturation brought about by a standardized heat treatment (Figure 4) is similar to that derived by Scheraga (5), in his consideration of the denaturation of a hypothetical protein stabilized by carboxyl-carboxyl hydrogen bonds.

References

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